Update on ECVAM's activities

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VALIDATION

COMPLETED VALIDATION STUDIES

- Carcinogenicity

  Pre-validation study on 3 Cell transformation assays (SHE pH 6.7, SHE pH 7, Balb/c 3T3) **- ESAC opinion delivered by written procedure, ECVAM preparing its draft recommendation, to be shared with ICATM before public consultation. ICCVAM consulted its own working group in parallel**

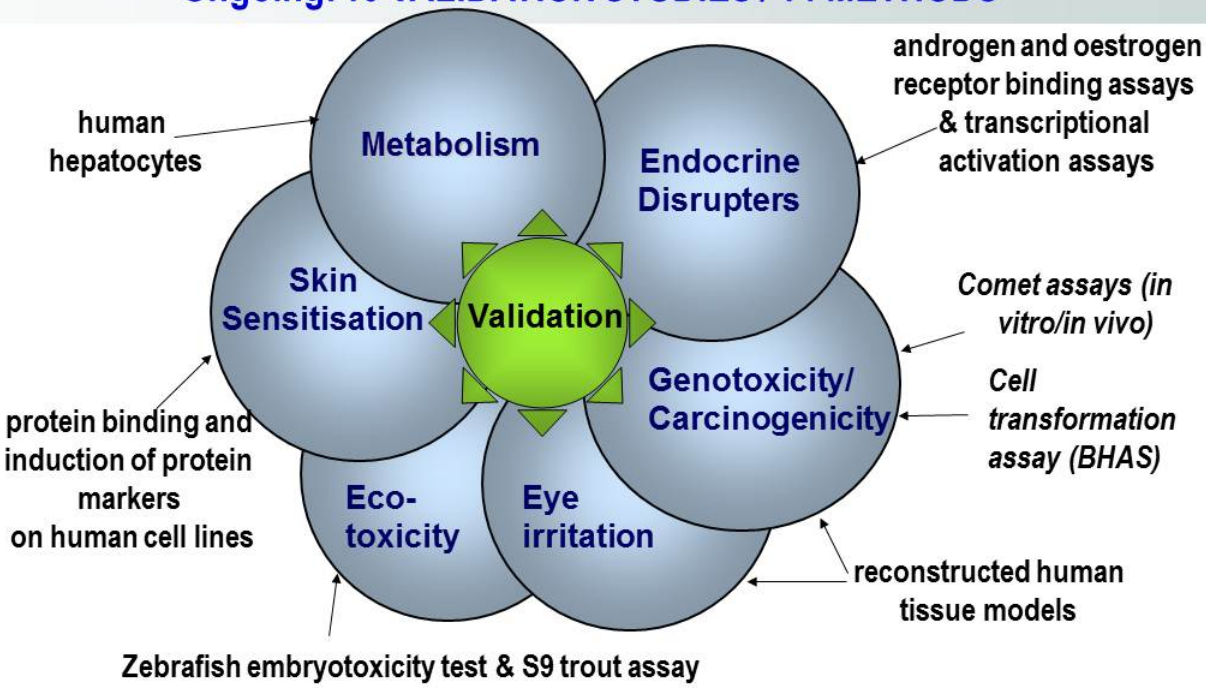
- Acute toxicity

  In vitro cytotoxicity test (3T3 Neutral Red Uptake) for identifying substances with acute oral LD50 > 2g/kg bw **– Request for ESAC opinion at ESAC 34 (22-23 March 2011), report available to ICATM via ESAC observers, discussion at ESAC 35 (3-4 Oct 2011)**

- Skin sensitisation

  DPRA & Keratinosens expected to be ready in 2011- **ESAC WGs were established at ESAC 34, opinion targeted for ESAC 35 – 36, reports not yet available (possible delay)**

Ongoing: 10 VALIDATION STUDIES / 14 METHODS



ECVAM-LED VALIDATION STUDIES – EYE IRRITATION (EIVS)

Objective: stand-alone test methods to identify chemicals not classified as eye irritant under GHS for use in a bottom-up testing strategy

Test systems: EpiOcular™ EIT and SkinEthic™ HCE

Status:

- 104 chemicals selected and undergoing testing in 3 laboratories
- Testing phase to finish tentatively in July 2011
- Analysis of data thereafter, Validation Report possibly to ESAC for peer review in March 2012

Note: The test methods are not intended to differentiate between GHS Category 1 (irreversible effects) and 2A-B (reversible effects). This differentiation would be left to another tier of the Bottom-up/Top-down testing strategy (ECVAM Workshop 2005; Scott et al., 2009).

ECVAM-LED VALIDATION STUDIES – EIVS: details

- A total of 104 chemicals selected from over 140 eligible chemicals
  - 45-55% split for irritants (UN GHS Category 1, 2A, and 2B) versus 'non-irritants' (UN GHS No Category)
  - 40-60% split for physical form (solids versus liquids)
  - 35-65% split for chemical reactivity (reactive versus non-reactive) based on EPRA
  - ± 50% split between GHS Category 1 and GHS Category 2 chemicals
  - Proper representation of Category 2A and Category 2B chemicals
- Study conducted in 3 phases
  - First set (32 chemicals) selected in June 2010, 2nd set (45 chemicals) selected in Sept. 2010, third and final set (27 chemicals) selected in April 2011
- SkinEthic™ HCE training and transferability in April 2010 and beginning of testing in June 2010 (1st chemical set)
- EpiOcular™ EIT training and transferability in October-November 2010 and beginning of testing in January 2011 (1st chemical set)

ECVAM LED VALIDATION STUDIES – SKIN SENSITISATION

Assessment of the reliability and preliminary evaluation of the predictive capacity of three skin sensitization test methods:

- **Direct Peptide Reactivity Assay (DPRA, Procter & Gamble).** Protein binding is a key step in the induction of skin sensitisation, this test uses HPLC to monitor a chemical's potential to deplete a nucleophile-containing synthetic peptide.

- **Human Cell Line Activation Test (h-CLAT, Kao and Shiseido).** This test monitors, using flow-cytometry, the induction of two protein markers on the surface of a human monocytic leukemia cell-line, following exposure to the chemical.

- **Myeloid U939 Skin Sensitization Test (MUSST, L'Oréal).** This test monitors, using flow-cytometry, the induction of a protein marker on the surface of a human dendritic cell like cell-line, following exposure to the chemical.

ECVAM LED VALIDATION STUDIES – SKIN SENSITISATION

Study Objective, concerning three assays: DPRA; h-CLAT; (MUSST)

Primary Goal:

- Assess the reliability (transferability and within & between laboratory reproducibility) of the 3 test methods
  - by challenging with a set of 24 coded chemicals (with known sensitisation profile)
- Request a peer review based ESAC opinion on the reliability of these tests

Secondary Goals:

- To perform a preliminary assessment of the ability of the tests to:
  - Discriminate skin-sensitising from non skin-sensitising chemicals
  - Categorise skin-sensitising chemicals into GHS sub-categories 1A / 1B

STATUS OF STUDY PROGRESS

Test Method	Training	Transfer	Phase B1 (9 coded chemicals)	Phase B2 (15 coded chemicals)
DPRA	**Completed** (all laboratories)	**Completed** (all laboratories)	**Completed** (P&G, Ricerca) **Ongoing** (IVMU)	**Ongoing** in two laboratories (P&G, Ricerca)
h-CLAT	**Completed** (all laboratories)	**Completed** (all laboratories)	**Ongoing** in all laboratories (Kao, Shiseido, Bioassay, IVMU)	
MUSST	**Completed** (all laboratories)	**Ongoing**		

GENOTOXICITY; COLIPA-led validation study

Study Objective:

Pre-validate the micronucleus test and the comet assay in reconstructed human epidermis models (*ECVAM involved in steering committee, sponsoring one lab & statistical support)*

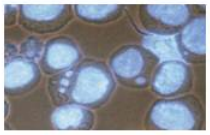
Test System: EpiDerm™

Study Organisation:

- **Phase I: Optimisation/transferability**
- **Phase II: Reproducibility**
- **Phase III: Further reproducibility and preliminary predictive capacity**
- **Testing phase finished in April 2011**
- **Analysis of data ongoing**

GENOTOXICITY; COLIPA-led validation study

Micronucleus test

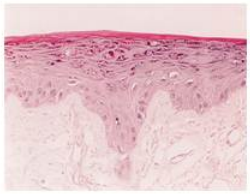


Phase I: 3 chemicals > completed

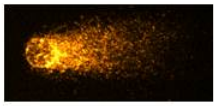
Phase II: 6 chemicals > completed

Phase III: 29 chemicals > completed

Reconstituted skin model



Comet assay



Phase I: 3 chemicals > completed

Phase II: 6 chemicals > completed

Phase III won't be completed due to technical difficulties related to stability of the Epiderm model

ECVAM-LED VALIDATION STUDIES - METABOLISM

Study Objective:

- Validate a cytochrome P-450 induction-based metabolic-competent model system (cryoHepaRG® cell line or cryo-preserved human hepatocytes)

Primary Goal:

- Assess transferability / reliability (within & between lab reproducibility) of the 2 model systems by challenging them with 12 coded chemicals

Secondary Goal:

- Perform a preliminary assessment of the ability of the test methods to:
  - **compare *in vitro* human CYP induction at clinically relevant doses to *in vivo* CYP induction data obtained from humans**
  - **categorise chemicals into CYP-inducers and non-inducers**

ECVAM-LED VALIDATION STUDIES - METABOLISM

State of play:

- **Training Phase successfully finalised.**
- **Experimental design and chemical selection ready**
  - first set of 4 coded chemicals aliquoted, coded and distributed
  - solubility phase finalised by all test facilities
- **CryoHepaRG & cryohepatocyte methods transferred to test facilities**
  - two confirmatory runs being finalised based on SOPs that were updated as a result of feedback by the transfer test facilities
- **CYP induction test method: final SOPs for blind coded testing phase**
  - agreement with VMG and test facilities on timelines to update the project plan
  - details on equipment specification added, e.g. for the LC/MS analysis

ECVAM-LED VALIDATION STUDIES – REPRODUCTIVE TOXICITY

Test method: MELN - Oestrogen Receptor (ER) - Transcriptional Activation Assay based on "MELN Cells"

Study Objective: To assess the method in view of a future incorporation into a testing strategy for detecting endocrine active compounds

- Establish transferability and reliability of the method through preparing a 3rd dataset by challenging the assay with the same 16 chemicals previously used, now blinded, with known estrogen receptor activation profile
- Prepare a preliminary assessment of the ability of the test methods to rank chemicals according to their potency for estrogen receptor activation or suppression by calculating their relative agonistic/antagonistic activity (RAA)
  - Positive control agonist:: Estradiol; Postive control antagonist: 4OH-Tamoxifen
  - Test chemicals: 12 agonists and 10 antagonists, 6 both
- Compare an additional non-blinded trial with the blinded trial to assess the impact of blinding on results

ECVAM-LED VALIDATION STUDIES - ECOTOXICITY

Zebrafish embryo toxicity test (OECD project 2.7);

ECVAM coordinated

Study objective:

Assess the reliability (transferability, within- and between-laboratory reproducibility) of the test method

Study status:

Phase 1 (protocol transfer & testing of 7 chemicals):

- finalised & report approved by OECD WNT in April 2011
- results (3 runs/chemical, in at least 3 laboratories) indicate good reproducibility (WLV CV < 20%; BLV < 30%)

Phase 2 (testing of 13 chemicals):

- started in January 2011 and will be finished in autumn 2011

ECVAM-LED VALIDATION STUDIES - ECOTOXICITY

In vitro trout S9 assay for fish bio-concentration testing

Study objective:

Assess the reliability (transferability, within- and between-laboratory reproducibility) of the method

Status:

- ECVAM and CEFIC co-funded
- Laboratory part finalized in June 2010
- Evaluation of results ongoing (in collaboration with HESI)
- Decision on how to continue outstanding

REGULATORY ACCEPTANCE

REGULATORY ACCEPTANCE

Approved by OECD since October 2010

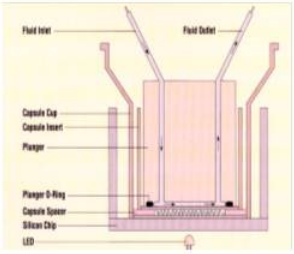
- Reduced Local Lymph Node Assay for skin sensitisation
- ICCVAM-ECVAM-JaCVAM harmonised LLNA Performance Standards
- TG 439 on 3 *in vitro* skin irritation tests (EpiSkin, EpiDerm SIT, SkinEthic RHE)

TGs proposed by ECVAM to WNT 23 in April, 2011

2 Cell-based assays for eye irritation

Fluorescein Leakage assay for the identification of ocular corrosives and severe irritants

Cytosensor Microphysiometer assay for the identification of ocular corrosives and severe irritants as well as "non-irritants" (limited applicability domain)



Draft OECD TGs

- *to ICATM 18th June 2010, to OECD end June 2010*
- commenting rounds: July and December 2010
- FL-TG provisionally approved by WNT23 pending inclusion of a list of Proficiency Chemicals
- CM-TG to be further discussed by OECD expert group

SPSF SUBMITTED TO OECD TO UPDATE TG 437 (BCOP)

Proposal to allow the use of BCOP for the identification of UN GHS/EU CLP "non-irritants"

- ICCVAM peer review panel concluded that BCOP can be used to identify chemicals not requiring a classification for eye irritation under UN GHS – and ECVAM agrees
- ECVAM wanted to get this into the work plan of OECD
- ECVAM has asked a CRO to re-test one doubtful substance (L-Aspartic acid) that was confirmed to be correctly identified as irritant by BCOP
  - The 0% false negatives for GHS/EU CLP is thus confirmed

TEST SUBMISSIONS

Preliminary SPSF on the Rat Recombinant Androgen Receptor Binding Assay submitted to OECD in January 2010

- No follow-up so far.
- Pre-submission received and assessed, full submission (to be) invited
- Consultation of PARERE (EU-Member States Regulatory Authorities) and ESTAF (ECVAM STAkeholder Forum) in the near future

Full Test Submissions (2010-2011)

- Skin sensitisation (1 test method)
- Endocrine Disruption (2 test methods, *1 PARERE/ESTAF*)
- Neurotoxicity (1 test method; *PARERE/ESTAF*)

Total: 4 test methods

Test Pre-submissions (2010-2011)

- Reproductive toxicity (1 test method, *PARERE/ESTAF*)
- Neurotoxicity (1 test method)
- Endocrine Disruption (1 test method)
- Eye Irritation (2 test methods)
- Skin irritation (1 test method)
- Genotoxicity (1 test method, *PARERE/ESTAF*)
- Cardiotoxicity (1 test method)
- Acute toxicity? (1 test method)

Total: 9 test methods

Test Pre-submissions (2008-2009) already followed by invitation to prepare a full Test Submission

- Genotoxicity (1 test method)
- Skin absorption (1 test method)
- Eye Irritation (1 test methods)
- Endocrine Disruption (1 test method)

Total: 4 test methods

OTHER ACTIVITIES

2013 marketing ban deadline under the Cosmetics Directive

ECVAM, together with 39 stakeholder-nominated experts, produced a technical report summarising the status and prospects of alternative methods for the endpoints of

- repeated-dose toxicity (incl. skin sensitisation and carcinogenicity),
- toxicokinetic,
- reproductive toxicity

Report was published in ***Archives of Toxicology*** and on the Website of the European Commission

2013 marketing ban deadline under the Cosmetics Directive

Arch Toxicol (2011) 85:367–485
DOI 10.1007/s00204-011-0693-2

REVIEW ARTICLE

Alternative (non-animal) methods for cosmetics testing: current status and future prospects—2010

Sarah Adler · David Basketter · Stuart Creton · Olavi Pelkonen · Jan van Benthem · Valérie Zuang · Klaus Ejner Andersen · Alexandre Angers-Loustau · Aynur Aptula · Anna Bal-Price · Emilio Benfenati · Ulrike Bernauer · Jos Bessems · Frederic Y. Bois · Alan Boobis · Esther Brandon · Susanne Bremer · Thomas Broschard · Silvia Casati · Sandra Coecke · Raffaella Corvi · Mark Cronin · George Daston · Wolfgang Dekant · Susan Felter · Elise Grignard · Ursula Gundert-Remy · Tuula Heinonen · Ian Kimber · Jos Kleinjans · Hannu Komulainen · Reinhard Kreiling · Joachim Kreysa · Sofia Batista Leite · George Loizou · Gavin Maxwell · Paolo Mazzatorta · Sharon Munn · Stefan Pfuhler · Pascal Phrakonkham · Aldert Piersma · Albrecht Poth · Pilar Prieto · Guillermo Repetto · Vera Rogiers · Greet Schoeters · Michael Schwarz · Rositsa Serafimova · Hanna Tähti · Emanuela Testai · Joost van Delft · Henk van Loveren · Mathieu Vinken · Andrew Worth · José-Manuel Zaldivar

Received: 21 February 2011 / Accepted: 3 March 2011 / Published online: 1 May 2011

Main findings

Current status:

- Full replacement alternative test methods/approaches will not be available by 2013
- No specific timeline could be estimated in the areas of toxicokinetics, repetead dose toxicity, carcinogenicity and reproductive toxicity (underlying scientific challenges)
- The timelines estimated for full replacement of animal tests in the area of skin sensitisation point to a further 7-9 years (i.e. 2017-2019), including the possibility to differentiate weaker from stronger sensitisers. Alternative methods able to simply discriminate between skin sensitisers and non-sensitisers might become available earlier.
- The forecasts for the full availability of alternative test methods made in the 2010 report do not diverge much from estimates provided in a similar review already conducted by the Commission in 2005

Main findings

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EFPIA/ECVAM post-validation WS on 3T3 NRU for phototoxicity

Since 2002, EMA guidance to use 3T3 NRU-PT in Pharma

- High rate of false positives with non-topical compounds

WS outcome: Ways to improve the 3T3 NRU-PT protocol

- testing only compounds showing a Molar Extinction Coefficient (MEC) > 1000 L/mol/cm.
- limit top concentration under irradiation to 100 µg/mL, and to consider higher top concentration without irradiation only to establish IC50 values for Photo Irritation Factor (PIF) calculation (if needed).
- to apply PIF < 5 threshold for "negative" results more generally (according to validation data), rather than PIF < 2
  - Requires further data review – industry is working on it

New Directive 2010/63 on the protection of laboratory animals

- The principle of the three Rs (replacement, reduction, refinement) no enshrined in EU-legislation
- ECVAM as EU Reference Laboratory
- EU Member States to nominate suitable and specialized labs for Validation Studies – network being set-up by ECVAM
- EU-MS nominated single points of contact for the validation of alternative methods – PARERE network managed by ECVAM
- ECVAM may charge fees for catch-up validations – conditions need to be established
- ECVAM shall promote use of alternatives also in basic research

ECVAM Advisory Structure

- ESAC established – first opinion Feb. 2011 (CTA)
- ECVAM Stakeholder Forum (ESTAF): over 30 applications, 15 eligible – first meeting 27 May 2011; stakeholder organisations with EU outreach; IND, NGO, Academic
- Network with the Member State single points of contact for preliminary assessment of regulatory relevance (PARERE) - first requests for a preliminary assessment of the regulatory relevance of submitted test methods discussed at first meeting 26 May 2011
- Network of suitable laboratories for validation – being set up 2011

DNT3 Developmental Neurotoxicity

Third International Conference on Alternatives for Developmental Neurotoxicity (DNT) Testing, May 10-13, 2011

"Advancing the science of developmental neurotoxicity testing for better safety evaluation"

Venue: Centro Congressi Ville Ponti, Varese, **Italy**

Website: http://ihcp.jrc.ec.europa.eu/dnt3conference/index.htm

Thanks for your attention!